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The Impact of Time Post Cardiac Transplant on Gene Expression Profile Scores, an Analysis of 32,043 Tests

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Abstract

Background: Gene Expression Profiling (GEP) has been used since 2005 to identify Orthotopic Heart Transplant (OHT) recipients at low risk of allograft rejection. A rise in GEP scores during the first year post OHT has been previously reported. The purpose of this study is to confirm and better characterize this effect of time post transplant in a larger, unselected cohort.

Methods: All commercially obtained GEP scores (XDx Inc., Brisbane, CA) collected between January 2005 and September 2011 were included. Clinical data were available for patient age, gender and dates of transplantation and tests.

Results: There were 32,043 GEP tests from 9,272 patients at 108 transplant centers (mean age at testing 54.2 ± 14.7 yrs, 25% women). There was a significant effect of time post transplant on GEP, which rose from 24.7 ± 7.8 at 2-6 months to 28.8 ± 5.8 at 7-12 months ($p<0.0001$) and 29.6 ± 5.0 ($p<0.0001$) at 13-24 months, after which it remained stable.

Conclusions: A rise in GEP scores is seen during the first year following OHT, which parallels the time frame of decreased corticosteroid dosing. Further study of this test characteristic may better inform clinical use of GEP testing in conjunction with endomyocardial biopsy.

Keywords: Cardiac transplant; Gene expression profile; Rejection

Abbreviations: GEP: Gene Expression Profile; OHT: Orthotopic Heart Transplant; EMB: Endomyocardial Biopsy; TPT: Time Post Transplant

Introduction

Allograft rejection is most frequently encountered during the first year after Orthotopic Heart Transplantation (OHT) and is one of the most common causes of early mortality. Unlike renal and liver transplantation, potential blood test indicators of rejection such as troponin I and B type natriuretic peptide have not demonstrated sufficient specificity to accurately identify acute rejection episodes following OHT in a clinically meaningful way [1-3]. As such, Endomyocardial Biopsy (EMB) has been the gold standard for rejection surveillance and diagnosis, but the invasive nature of this modality is associated with a small risk of serious complications, such as bleeding, arrhythmias, pneumothorax, and tricuspid valve damage [4,5]. Gene Expression Profiling (GEP [AlloMap®], XDx, Inc., Brisbane, CA) is a noninvasive method of rejection surveillance developed and validated in the CARGO and CARGO II studies [6,7]. The test score is based on the relative expression of a panel of 11 informative genes involved in 7 pathways, with an additional 9 genes for quality control and standardization. Three of the 11 informative genes are corticosteroid-responsive. The IMAGE (Invasive Monitoring Attenuation through Gene Expression) study is a prospective, randomized, multi-center study that included 602 OHT patients and demonstrated that rejection surveillance using GEP was non-inferior to EMB with respect to clinical outcomes in stable patients at least six months following OHT. GEP scores range from 0 to 40, and the IMAGE study used a reference value of 34 or above to prompt further investigation of possible acute cellular rejection [8,9]. The negative predictive value using this reference value in the CARGO validation study exceeds 98% [6]. GEP testing is used clinically to aid in the identification of OHT recipients with stable allograft function who have a low probability of moderate to severe acute cellular rejection. This test has been commercially available since January 2005 and is cleared by the Federal Drug Administration for use in patients age 15 and older at least 55 days post OHT.

A rise in GEP scores has been reported during the first year following OHT in 243 samples evaluated in early clinical use of the test [10]. Postulated explanations for the rise in scores over time despite lower overall risks of rejection have included immune activation through pathways related to chronic allograft vasculopathy, diminished immunosuppression with corticosteroid agents, and infection [11-13]. The purpose of this study is to confirm and further characterize the trend in GEP scores by Time Post Transplantation (TPT) in a large unselected cohort of OHT in order to further inform clinical use.

Materials and Methods

Data

This study included all commercially obtained GEP test scores between January 2005 and September 2011. Tests used for validation of GEP in the CARGO study and those samples used for other clinical research studies were excluded from this analysis. All tests were obtained from patients at least 55 days post OHT and ≥ 15 years of

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age. Since these tests were commercially obtained, available clinical variables were limited to medical centers, TPT, age, and gender of the patients. Other information, including immunosuppressive dosing, biopsy results, cytomegalovirus serostatus, allosensitization, or etiology of cardiomyopathy, was not available. Procedures for data collection were in accordance with the Helsinki Declaration of 1975.

Statistical methods

The TPT was divided into five time periods in the analyses: period 1 = 55 days to 6 months, period 2 = 7 to 12 months, period 3 = 13 to 24 months, period 4 = 25 to 60 months, and period 5 = greater than 60 months post transplant. Student's t test was used to compare GEP scores at the different periods post transplant.

Results

A total of 32,043 GEP tests were obtained from 9,272 patients at 108 transplant centers between 1/2005 and 9/2011. The mean age at the time of testing was 54.2 ± 14.7 years, with 25% of the patients tested being women. Patient population and test characteristics are described in Table 1A and 1B. The majority of patients (75.2%) were between 18-65 years of age. Mean overall GEP score is 28.8, and there were a mean of 3.5 tests per patient. The distribution of tests by TPT was 14.7% in period 1, 14.6% during period 2, 14.8% during period 3, 21.3% during period 4, and 34.6% during period 5. As illustrated in Figure 1, 78.1% of the GEP test scores were below the reference value of 34. Figure 2 illustrates the relationship between GEP scores according to TPT. Test scores for patients more than 6 months from OHT are shown in grey bars, whereas those scores of patients less than 6 months from OHT are shown in black bars. For those patients less than 6 months from OHT, 89% of GEP scores were below 34, whereas 77% of scores of patients more than 6 months post OHT were less than 34. The 5th percentile of GEP scores was 13.1, while the 95th percentile of GEP scores was 37.6.

The rise in scores after the first year following transplant is graphically described in Figure 3. Time post transplant had a significant effect on GEP scores, which rose from 24.7 ± 7.8 during period 1 to 28.8 ± 5.8 during period 2 ($p<0.0001$), then to 29.6 ± 5.0 ($p<0.0001$) during period 3, after which they remained stable. Mean scores at discrete time points were also consistent with this trend. Mean GEP score was 21 (CI: 20.4-22.0) at 2 months, which rose to 27 (CI: 26.3- 27.1) at 6 months, more slowly to 29 (CI: 28.9-29.7) at 12 months, and then plateaued at 30 (CI: 29.1- 29.8) at 24 months. The steady rise in scores over the first 12 months following OHT from 21 to 29 was statistically significant ($p<0.001$).

Total tests	32,043
Total patients	9272
Total centers	108
Men	6933
Age	54.2 ± 14.7
Mean tests / patients	3.5
Median tests / patients	2
Mean GEP score*	28.8 ± 6.0
Median GEP Score*	30

*GEP: Gene expression profile

Table 1A: Patient demographics and data description of 32,043 commercially obtained gene expression profiling tests.

Age Range	% Total Population
15-18	2.2
19-35	11.2
36-50	18.1
51-65	45.9
>65	22.6

Table 1B: Distribution of gene expression profile tests by age.

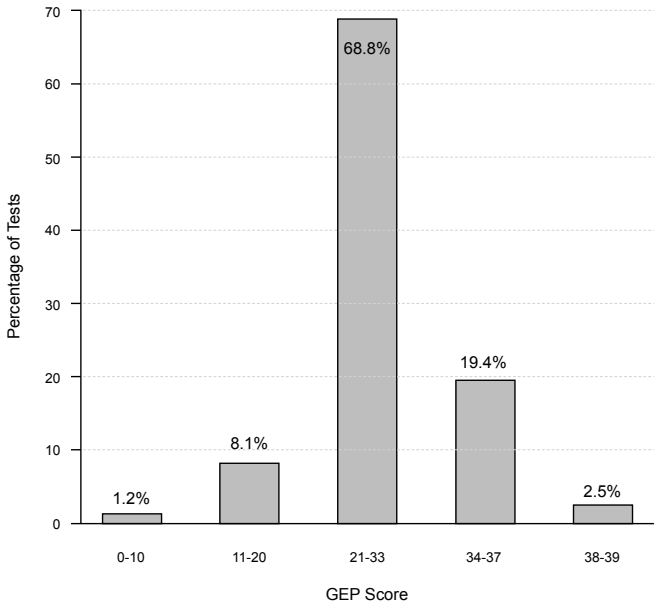
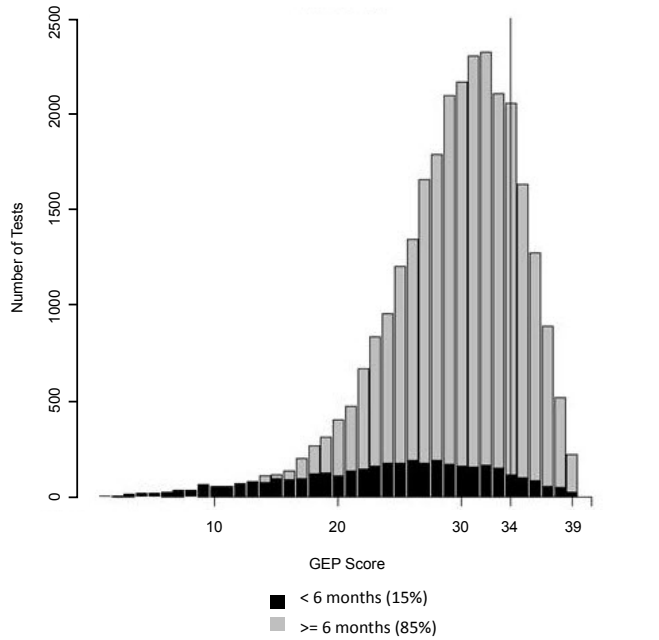


Figure 1: Distribution of gene expression profile scores (n=32,043). GEP=gene expression profile.



Month Post Transplant	Percent of Tests with GEP score < 34
< 6 months	89%
≥ 6 months	77%

Figure 2: Differential distribution of gene expression profile scores<6 months and>6 months post transplant. GEP=gene expression profile.

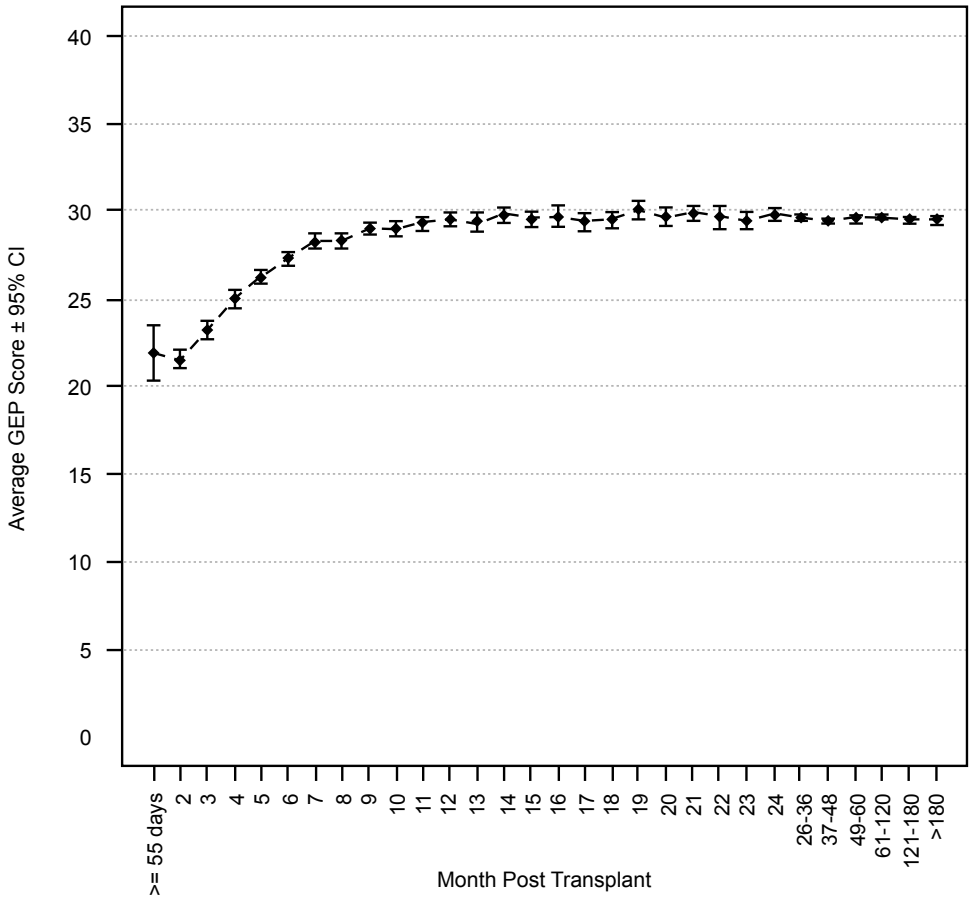


Figure 3: Rise in gene expression profile (GEP) scores by time post transplant in 32,043 commercially obtained GEP tests. The error bars are confidence intervals. GEP=gene expression profile.

Discussion

In this retrospective analysis of 32,043 commercially obtained GEP tests, 25% were obtained in women, fifty-one percent of tests were obtained between 6 months and 5 years post OHT consistent with the time period evaluated in the IMAGE study, and almost 30% were in the first year following OHT, the time of greatest risk for rejection. Overall mean GEP score was 28.8, and 90% of scores were between the values of 13.1 and 37.6. As 78% of scores are less than the reference value of 34, this suggests that many patients may potentially avoid undergoing a routinely scheduled surveillance EMB.

This study confirmed that TPT has a significant effect on GEP scores during the first year following OHT, with scores rising most quickly in the first six months from an average of 21 at 2 months to 27 at 6 months, followed by a more gradual increase to 29 at 1 year post OHT. Thereafter, GEP scores remained stable at 30 beyond 15 years post OHT. This rapid rise in scores during the first 6 months post transplant has implications for reference values during that time period. The IMAGE study used a reference value of 34 to prompt further evaluation of possible acute cellular rejection between 6 months and 5 years post OHT [10]. Initial experience following the introduction of GEP testing suggested that scores of less than 20 in patients 2 to 6 months after transplant, less than 30 in patients 6 to 12 months post transplant, and less than 34 in patients greater than 12 months post transplant were felt to represent a very low risk of acute cellular rejection as detected on EMB [12]. Previously published data estimated a negative (NPV) and Positive Predictive Value (PPV) of 97.1% and 8.8%, respectively, for ISHLT grade ≥ 3A/2R rejection using a reference value of 34 at less than 6 months following OHT, and NPV of 97.5% and PPV of 6.2% using a reference value of 30 during that time frame [12]. Looking at the 3,712 commercially available GEP test scores obtained at less than 6 months post OHT, 88% were below 34 and 71% were below 30. Given the marked rise in scores during the first 6 months post OHT, a lower reference value of 30 may be warranted during that time period to reduce the number of potential "false negative" results in light of the increased risk for rejection in that time frame. Future and ongoing studies such as the CARGO II study (7) and E-IMAGE (http://clinicaltrials.gov/ct2/show/NCT00962377?term=allomap&rank=1) may provide additional evidence to further refine and potentially personalize selection of appropriate reference values between 55 days and 6 months post transplant.

Study limitations

As the test results analyzed were limited to commercially ordered tests, clinical variables such as including immunosuppression, corticosteroid dosing, allosensitization, CMV serostatus, etiology of cardiomyopathy, biopsy results and outcomes were not available. As such, it is possible the results may be biased by potential unknown imbalances in factors that could affect the GEP scores (e.g. the actual doses of corticosteroid in the different time periods

are unknown). Another potential source of bias may come from over or underrepresentation of the percentage of patients who were cytomegalovirus serology negative in both donor and recipient which is known to influence GEP scores.

The scores represent a cross-sectional composite that is not adjusted for the number of tests per patient or timing of tests from any one individual. However, since this study includes a relatively large sample population (9,272), it is reasonable to assume the results are representative of the total heart transplant population (approximately 20,000 recipients surviving in 2013). The average age and proportion of men and women in this study cohort is well matched to the demographics of the total heart transplant population reported in the Scientific Registry of Transplant Recipients Annual Data reports for 2011.

Conclusions

A notable characteristic of commercially available GEP testing is a progressive rise in scores over the first year following OHT, which then stabilizes. This is consistent with a prior report of the initial clinical experience and parallels the time frame of decreased corticosteroid dosing in many transplant centers [12]. Considering this expected rise in GEP scores and the higher potential risk for rejection in the first year post OHT, a lower reference value such as 30 during the first 6 months post OHT may be appropriate. The stability of average scores after the first year post OHT confirms the appropriateness of current clinic practice using the standard reference value of 34. Further study of GEP testing as it relates to outcomes may better inform clinical use, particularly as an instrument to facilitate more individualized approaches to strategies for immunosuppression and cellular rejection surveillance.

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